

PRISTINA PROBOSCIDEA AND *PRISTINELLA OSBORNI* (OLIGOCHAETA,
NAIDIDAE) FROM A FRESHWATER CREEK
NEAR DARWIN, NORTHERN TERRITORY, AUSTRALIA,
WITH DESCRIPTIONS OF THE GENITAL ORGANS
OF BOTH SPECIES

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ABSTRACT

Naidid oligochaetes from a freshwater creek feeding into West Arm, Darwin Harbour, were collected in July 1993. The majority of the worms were in different stages of sexual maturity. They represent two species, *Pristina proboscidea* Beddard, 1896, and *Pristinella osborni* (Walton, 1906). In this paper, full descriptions of both somatic and genital features of these taxa are given. Taxonomic and phylogenetic aspects are discussed.

KEYWORDS: Naididae, Oligochaeta, *Pristina*, *Pristinella*, taxonomy, zoogeography

INTRODUCTION

The Naididae, with a total of about 175 species in about 25 genera worldwide, comprise a considerable part of the freshwater oligochaete fauna of all continents except Antarctica. Several genera and species of this family are more or less cosmopolitan, occurring in virtually all kinds of freshwater habitats. There is a surprising coincidence in the number of species found on the different continents: 54 species are known from Europe, 81 from Asia, 51 from Africa, 55 from North America, and 52 from South America (Brinkhurst and Jamieson 1971; Brinkhurst 1986; Harman *et al.* 1988; Grimm 1987; Semernoy and Timm 1994). The high score of Asian species mainly depends on the fact that Lake Baikal, with its many endemic taxa, has been extensively studied by Russian workers (Semernoy and Timm 1994). Some of these species are, however, briefly described. A recent compilation of

records of microdrile Oligochaeta from inland waters of Australia (Pinder and Brinkhurst 1994) includes 31 species of Naididae, but it can be expected that this number will increase when additional habitats have been investigated.

The wide distribution of the Naididae is largely due to their opportunistic strategy of asexual reproduction as the normal case. For many naidid species, reproductive organs have never been described, which is unsatisfactory, as the classification and phylogenetic assessment of oligochaetes are largely based on the position and morphology of these structures.

While participating in the Sixth International Marine Biological Workshop on the Marine Flora and Fauna of Darwin Harbour, at Mandorah, near Darwin (Northern Territory, Australia) in July 1993, the first author collected a few naidid oligochaetes from a freshwater creek feeding into West Arm, Darwin Harbour. The majority of these worms were in different

stages of sexual maturity. The material represents two species, *Pristina proboscidea* Beddard, 1896, and *Pristinella osborni* (Walton, 1906), both widespread in the tropics, but never before reported as sexually mature. In this paper, full descriptions of both somatic and genital features of the two taxa are given, and some taxonomic and phylogenetic aspects of these findings are discussed.

Other material of (largely marine) oligochaetes collected during the Darwin Harbour workshop has been published separately (Coates and Stacey 1997; Erséus 1997; Healy and Coates 1997).

METHODS

The sand from the creek was repeatedly stirred with habitat water and the organic suspensions decanted into a fine-mesh sieve (250 µm). Live worms were sorted under a dissecting microscope and fixed in Bouin's fluid. After about one day, they were transferred into 70% ethanol. All specimens were stained in alcoholic paracarmine and mounted whole in Canada balsam. Material of both species is deposited in the Museum and Art Gallery of the Northern Territory (NTM). Some specimens of *Pristina proboscidea* are also lodged in the Swedish Museum of Natural History (SMNH), Stockholm.

In the descriptions, segment numbers are denoted by Roman numerals.

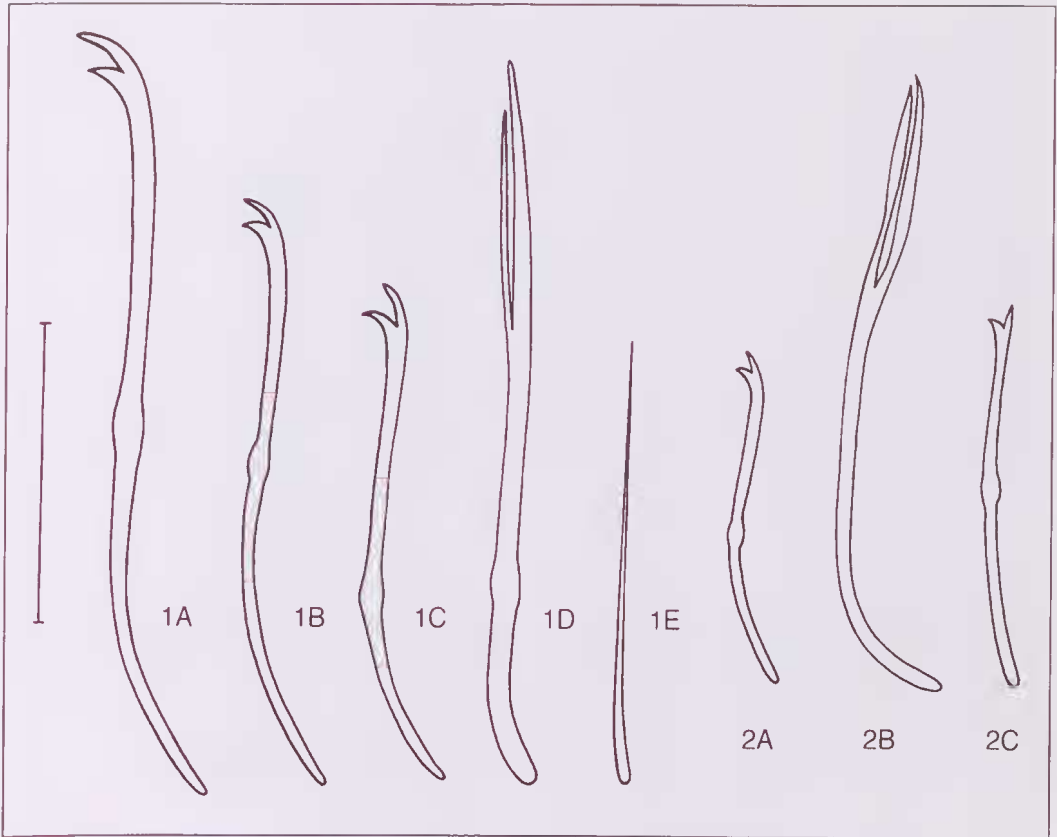
Pristina proboscidea Beddard, 1896 (Figs 1, 3)

Material studied. NTM Wo 0121–0127, 7 whole-mounted specimens, and SMNH Main coll. 1615–1618, 4 whole-mounted specimens; all from small freshwater creek feeding into Stephens Creek, West Arm, Darwin Harbour, Northern Territory, Australia, 12°38'S, 131°43'E, sand with roots and debris along a short stretch of pools and rapids; coll. C. Erséus, 15 July 1993 (CE station no. 35).

Description. Ten specimens in various stages of sexual maturity, and one immature chain with two zooids. Two complete

specimens (one mature, plus the immature worm) both 3.5 mm long (contracted), consisting of a total of 46 and 35 segments, respectively. Anterior zooid of immature worm with 21 segments. Prostomium with proboscis (Fig. 3A). Clitellum extending over VIII–IX, when developed. Dorsal setae: hairs one to four per bundle, serrate, about 200 µm long, none especially elongated; needles one to four per bundle, straight, single-pointed with fine tips, and without nodulus, 42–49 µm long. Ventral setae three to seven per bundle in II–III, five to seven per bundle in IV, four to eight per bundle in V, up to seven per bundle in VI of asexual specimens, up to nine per bundle in following segments, decreasing to mostly five per bundle further towards the posterior. All setae bifid, with distal prong slightly longer than proximal (Fig. 1A–C). Ventral setae of II–V 54–60 µm long, posterior ventral setae 48–58 µm long. One specimen with ventral setae in II and III longer (63–78 µm) and thicker than the rest. In VI of specimens in an advanced stage of sexual maturity, ventral setae (genital setae; Figs 1D; 3B: gs) about 70 µm long, bifid with prolonged straight, parallel, teeth comprising one third of setal length, and with nodulus at one third from proximal end (Fig. 1D). One specimen of full sexual maturity with one slightly modified bifid seta representing each ventral bundle in VIII (Fig. 1C). In yet another mature specimen (depicted in Fig. 3B), ventral setae of VIII lacking. Male and spermathecal pores paired, in line with ventral setae; male pores anterior to middle of VIII, spermathecal pores immediately posterior to furrow between VI and VII.

Pharyngeal glands in III–V. Coelomocytes few, spherical, each with a distinct nucleus surrounded by weakly granulated cytoplasm. Transition from oesophagus to stomach inconspicuous, somewhere behind VII (marked by presence of gut content). Male genitalia (Fig. 3B) paired. Sperm funnel indistinct, position and shape (indicated in Fig. 3B) deduced from adhering spermatozoa and position of vas deferens. Vas deferens and atrium a continuous tubular male duct, at least about 375 µm long. Proximal end of male duct (Fig. 3B: vd), a short, naked part of vas



Figs 1–2. *Pristina proboscidea*: 1A, elongate ventral seta in segment II; 1B, normal posterior ventral seta; 1C, single ventral seta in VIII; 1D, genital seta in VI; 1E, dorsal needle seta. *Pristinella osborni*: 2A, posterior ventral seta; 2B, genital seta in VI; 2C, dorsal needle seta. Scale: 30 μ m.

deferens, up to about 15 μ m wide. Middle part of male duct, i.e. remaining part of vas deferens, embedded in continuous mass of tightly packed, heavily granulated, prostate cells (pr); this part of duct ciliated, somewhat coiled, but very difficult to follow throughout its length. Distal part of male duct (a) a naked atrium, about 170 μ m long, 12–18 μ m wide, terminally opening to the exterior through inconspicuous pore; atrium appears non-ciliated. No part of male duct with notable muscular layer. Spermathecae (Fig. 3B: s) small and club-shaped, each with duct, about 35 μ m long, and about 28 μ m wide, and an oval ampulla, about 60 μ m long, and about 45 μ m wide. In one specimen (Fig. 3B), spermathecal ampullae containing loose masses of stained material reminiscent of sperm, but these 'spermatozoa' much shorter than those adhering to sperm funnel.

Remarks. The first description of *Pristina proboscidea* was given by Beddard (1896). It was based on two South American sexually immature specimens and is rather incomplete. Sperber (1948) redescribed the species as completely as it was then possible. The specimens of the present study fit Sperber's description with the exception of slight differences in the number of ventral setae in the anterior segments, which, however, lie within the variability usual for naidids.

Pristina proboscidea has been considered to be closely related to the highly variable *P. longiseta* Ehrenberg, 1828 (e.g., Rodriguez 1987). To date, the only important taxonomic difference between these two taxa has referred to the possession of elongated hair setae in segment III in *P. longiseta*; in *P. proboscidea* the hair setae of this segment are as long as those of the neighbouring

segments. Typically, the hairs of both *P. longiseta* and *P. proboscidea* are serrated, but the elongate hairs of *P. longiseta* are normally smooth. Sometimes, however, the setal characteristics of *P. longiseta* overlap with those of *P. proboscidea*. In African material, Grimm (1990) found (1) specimens with elongate, smooth hair setae in segments II and III; (2) a large variation in the serration of the hair setae (even the elongate hairs in segment III were sometimes serrated), which has been used to separate subspecies in *P. longiseta* (Sperber 1948: 237; Brinkhurst and Jamieson 1971: 403); and (3) specimens in which only the hairs of III, or those of both II and III, were reduced in length or even completely lacking, i.e. animals that according to an identification key would be *P. proboscidea*. Absence of elongate hairs may in some cases be due to loss, probably in handling the specimens. Because of these taxonomic uncertainties, Grimm (1990) did not include *P.*

proboscidea in the list of African naidid species. Its possible African occurrence has been based on a single individual found on Zanzibar (Michaelsen 1905), but the affiliation of this specimen with *P. proboscidea* is more than doubtful. On the basis of setal morphology, the question may even arise whether *P. proboscidea* is merely a variety of the widespread *P. longiseta* with reduced hair setae in segment III.

However, the present material of *P. proboscidea* has revealed that the genital organs are different in the two taxa. In *P. longiseta*, the atria are clearly wider and have thicker walls than the vasa deferentia, and although the epithelia of both the atria and vasa deferentia are glandular, prostate glands are nowhere present (Piguet 1906: Figs 22, 24–25; Sperber 1948). This differs considerably from the slender, thin-walled atria, and heavily prostatic vasa deferentia in *P. proboscidea* (Fig. 3B). Furthermore, in *P. longiseta*, the spermathecae are not small

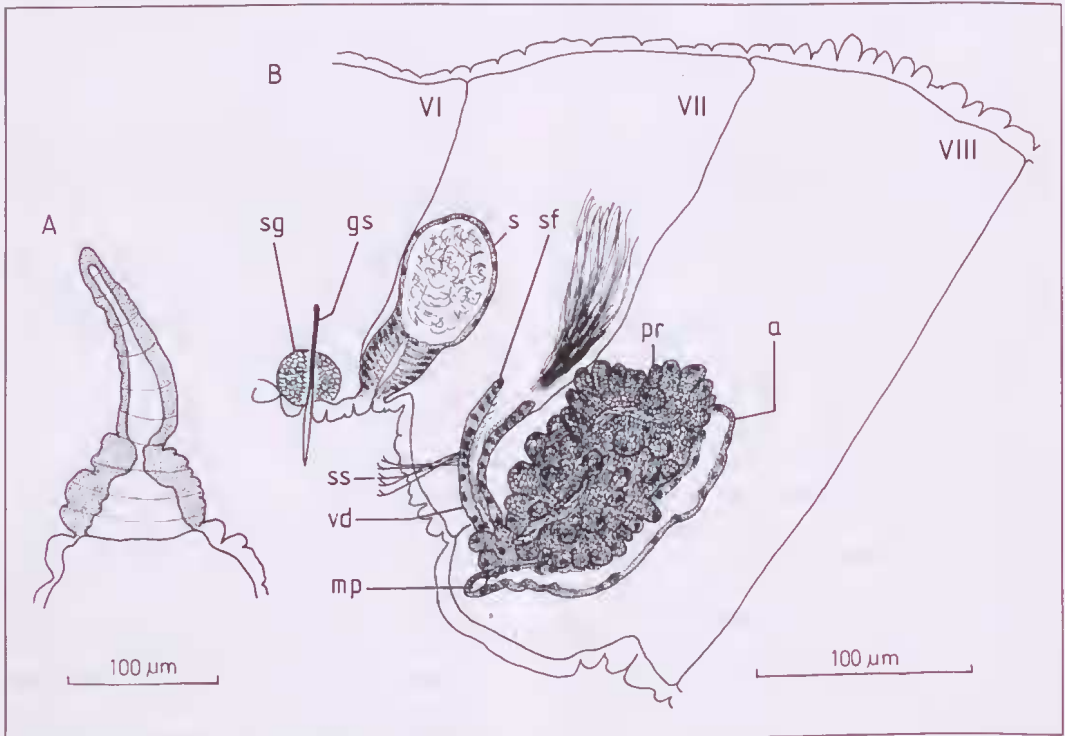


Fig. 3. *Pristina proboscidea*. A, prostomium with proboscis; B, lateral view of segments VI–VIII, showing genital seta, spermatheca and male duct of one side of worm. Abbreviations: a, atrium; gs, genital seta; mp, male pore; pr, prostate gland; s, spermatheca; sg, setal gland; se, somatic seta; sf, sperm funnel; vd, vas deferens.

and elub-shaped (as in *P. proboscidea*; Fig. 3B), but long, with ampullae extending through the whole length of segment VII (Piquet 1906: Figs 22, 25). Finally, Piquet reported that, in *P. longiseta*, the glands associated with the genital setae in VI do not envelop the setae but are situated separate from and behind these setae (Piquet 1906: Figs 22, 25).

With regard to the prostatic vasa deferentia and the slender, thin-walled atria, *P. proboscidea* resembles *P. americana* Cernovitov, 1937 (Cernovitov 1937: Fig. 10) more closely than *P. longiseta*.

Distribution. To date, *Pristina proboscidea* has been recorded from North (Brinkhurst 1986) and South America (Harman *et al.* 1988), South (Ali and Rashiduzzaman 1976) and East Asia (Brinkhurst *et al.* 1990), Australia (Pinder and Brinkhurst 1994; present paper) and New Zealand (Bayly 1989). Its occurrence in continental Africa is less certain (see above), but it has been reported from the irrigation system of Tenerife (Grimm 1978). A record from the Iberian Peninsula (Rodríguez and Armas 1983) is the only known European occurrence so far. The species thus appears widespread, but it may not be cosmopolitan. Rodríguez (1987) considered the special geographical situation of the Iberian Peninsula to favour recent introductions of American naidid species. This could apply also to Tenerife.

Pristinella osborni (Walton, 1906)
(Figs 2, 4)

Material studied. NTM Wo 0128. 1 whole-mounted specimen from small freshwater creek feeding into Stephens Creek, West Arm, Darwin Harbour, Northern Territory, Australia, 12°38'S, 131°43'E, sand with roots and debris along a short stretch of pools and rapids; coll. C. Erséus, 15 July 1993 (CE station no. 35).

Description. Length (contracted) 1.9 mm. Sexually mature specimen without budding-zone, with 26 segments. Prostomium without proboscis, rounded. Clitellum not developed. Dorsal setae: hairs one per bundle, smooth; needles, one per bundle,

bifid with nodulus at half to one third from distal end. Teeth of needles clearly visible, equally long (about 2.5 µm) and diverging at a wide angle (Fig. 2C). Needles 39 µm long in II, 34 µm in III, decreasing towards 30 µm in posterior segments. Ventral setae four to five per bundle in II, five per bundle in III–IV, six per bundle in V, one per 'bundle' in VI–VII, four per bundle in VIII, then decreasing to three per bundle posteriorly. Most anterior ventrals with distal tooth slightly longer than proximal, and nodulus proximal; posterior ventrals with equally long teeth and nodulus median to slightly distal (Fig. 2A). Ventral setae not measurable in anterior segments; setae more than 32 µm long, decreasing to about 30 µm in the posterior segments. In segments VI and VII, genital setae modified, about 60 µm long, without nodulus, bifid with prolonged straight, parallel, teeth comprising one third of setal length (Figs 2B, 4: gs). Each genital seta associated with large gland (Fig. 4: sg) histologically resembling prostate glands. Male and spermathecal pores paired, in line with ventral setae; male pores somewhat anterior to middle of VIII, spermathecal pores immediately posterior to furrow between VI and VII.

Pharyngeal glands in III–V. Coelomocytes numerous, spherical, about 5–10 µm wide, each with distinct nucleus surrounded by weakly granulated cytoplasm. Stomachal dilatation not distinct. Male genitalia (Fig. 4) paired. Sperm funnel indistinct, but revealed by adhering spermatozoa. Male duct a continuous, ciliated, tube, at least about 180 µm long, about 10–12 µm wide; transition between vas deferens and atrium not obvious. Proximal part of duct naked, middle part surrounded by continuous mass of tightly packed, heavily granulated, prostate cells. Distal part of male duct naked and terminally opening to exterior through inconspicuous pore. No part of male duct with any notable muscular layer. Spermathecae (Fig. 4: s, so) small and club-shaped, each with somewhat bulbous duct, about 20 µm long, about 15 µm wide, and oval ampulla, 30–35 µm long, about 25 µm wide. Spermathecal ampullae containing spermatozoa, somewhat loosely arranged in small bundles.

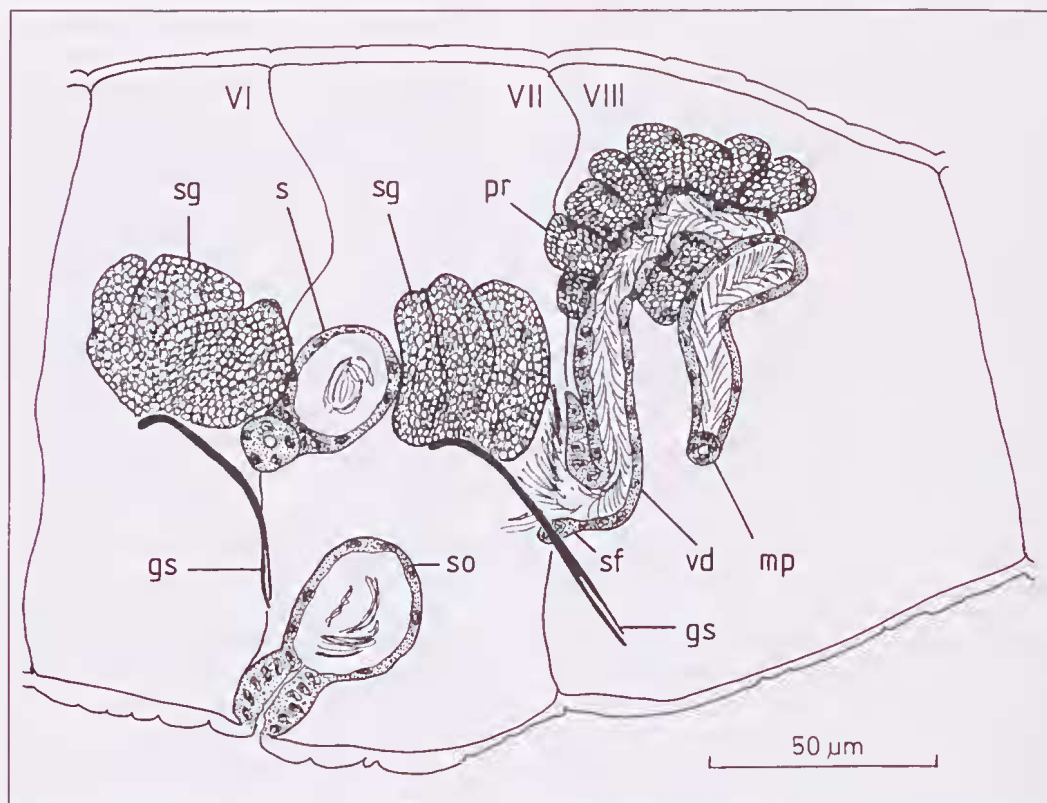


Fig. 4. *Pristinella osborni*, somewhat obliquely lateral view of segments VI–VIII, showing genital setae and male duct of one side of worm, plus spermathecae of both sides. Abbreviations: gs, genital seta; mp, male pore; pr, prostate gland; s, spermatheca; sf, sperm funnel; so, spermatheca of other side; vd, vas deferens.

Remarks. This species, one of the smallest naidids known, was first described by Walton (1906), as *Naidium osborni*, from Ohio, North America. Stephenson (1914) described *Naidium minutum* from Lahore, India. Sperber (1948) placed both species in the genus *Pristina* as *P. minuta* Stephenson, 1914. Brinkhurst and Jamieson (1971) stated that the separation of *Naidium osborni* from *N. minutum* was based on such slight differences that it had to be neglected, and correctly used the oldest name, *Pristina osborni* (Walton, 1906). Following Brinkhurst (1985), it is now called *Pristinella osborni* (see Discussion).

The new specimen fits the description given by Sperber (1948). Its needle setae are simply bifid and not pectinate. Intermediate teeth as reported by Grimm (1990) and Pinder and Brinkhurst (1994) are not visible (very few needle setae are in a suitable position for detailed observation).

According to Brinkhurst and Jamieson (1970), there should be a sudden stomachal dilatation in VII or VIII, but none is visible in the present material.

As described above, each male duct of *P. osborni* is a ciliated tube without a clear demarcation of an atrium, and it is not conclusive whether or not a proper atrium (an invaginated, ectodermal, part of the male duct) is present.

Distribution. *Pristinella osborni* appears more or less cosmopolitan. It is the most abundant *Pristinella* species in Europe, where it is often found in freshwater meiobenthos and in groundwaters. It has been reported from southern Europe (Martínez-Ansemil 1984; Sambugar 1986) up to the River Rhine basin in the north (Lafont and Durbec 1990; Wiegand and Matthess 1993). In America, the northernmost report comes from Lake Erie (Spencer 1977), the southernmost from

Bolivia (Juget and Lafont 1994). It is also recorded from Africa (Grimm 1990), Asia (Naidu 1961) and Australia (Pinder and Brinkhurst 1994; present paper).

DISCUSSION

Pristina Ehrenberg, 1828, and *Pristinella* Brinkhurst, 1985, originally treated as a single genus (*Pristina*), are currently the only genera in the subfamily Pristininae *sensu* Sperber (1948), a taxon subsequently revised to become a tribe, Pristinini, within the Naidinae (Nemec and Brinkhurst 1987). This group is 'curiously homogeneous' (Sperber 1948: 247), differing from all other Naididae by (1) its dorsal setae commencing from segment II, (2) its continuously tubular male ducts, and (3) the relatively high number (seven as opposed to four to five) of segments formed at the anterior end in a budding zone. At least the first two of these features may be plesiomorphic, but for the sake of the following discussion it is assumed that the Pristininae/Pristinini are monophyletic. Sperber's (1948) description of the Pristininae also mentions 'the characteristic stomach, with cells containing intra-cellular canals with special features', but these canals were not observed in the present material. As pointed out by Brinkhurst (1985), they need to be re-investigated using electron microscopy.

Brinkhurst (1985) separated *Pristinella* from *Pristina sensu stricto*, referring to a number of morphological differences. According to him, *Pristina* is characterised by a prostomial proboscis, genital setae in at least two different segments (varying positions), presence of spermathecae, and prostate glands on the vasa deferentia, whereas in *Pristinella*, proboscis, prostate glands and spermathecae are absent, and genital setae are either absent, or present in VIII (based on the genital organs of only two species, *Pristinella amphibiotica* (Lastockin, 1927), and *P. idrensis* (Sperber, 1948)). *Pristina proboscidea* fits into this general pattern, whereas *Pristinella osborni* does not. The latter possesses spermathecae as well as prostates, and it has genital setae in both VI and VII, but not in VIII (see Fig. 4).

Thus, with regard to genital features, *P. osborni* fits Brinkhurst's definition of *Pristina* better than that of *Pristinella*.

Pristina and *Pristinella* contain about ten species each, but they are often difficult to discriminate because of a wide overlap of taxonomic characteristics. Several additional nominal species in the literature are regarded as dubious (e.g., four species described by Botea 1983) or as junior synonyms of other taxa.

The male ducts are now known for six of the *Pristina* species: *P. longiseta* (type species), *P. breviseta* Bourne, 1891, *P. plumaseta* Turner, 1935, *P. leidy* Smith, 1896, *P. americana* and *P. proboscidea*. The atria are generally slender (short in *P. plumaseta*), and when present, the prostate glands are associated with the vasa deferentia, not with the atria. Prostates have not been observed in *P. longiseta* and *P. leidy*, but the inner ends of the male ducts have somewhat glandular walls in these two species (Piguet 1906; Smith 1896).

For the taxa currently placed in *Pristinella* (see Brinkhurst 1985), information on male ducts is scanty. According to Sperber (1948), *Pristinella amphibiotica* and *P. idrensis* have short vasa deferentia followed by atria that are either 'not differentiated' or 'small'; prostate cells are 'probably' absent. In his description of '*Pristina amphibiotica changtuensis*', Liang (1963: fig. 2F) illustrated a more developed and differentiated atrium, the male duct, however, still lacking prostate glands. *Pristinella osborni*, on the other hand, has prostate glands along the middle part of its male ducts but lacks demarcated atria (Fig. 4). In fact, as the male ducts of *P. osborni* are ciliated throughout, this species may lack atria altogether; Sperber (1948: 49) claimed that naeid atria are non-ciliated. The previously studied material of *P. amphibiotica* and *P. idrensis* were possibly not as sexually mature as the present individual of *P. osborni*, which could explain why prostate glands and spermathecae were not observed in the two former taxa. Alternatively, *P. osborni* could be regarded as representing a third (yet un-named) genus in the *Pristina/Pristinella* complex. At any rate, the distinction between *Pristina* and *Pristinella* does not appear as clear as it was initially thought to be (see Brinkhurst 1985).

A prostomial proboscis does not occur only in *Pristina*, but also in the other naidid genera *Stylaria* Lamarck, 1816, *Arcteonais* Piguët, 1928, and *Ripistes* Dujardin, 1842, and *Homochaeta* Bretscher, 1896 (Grimm 1985). The phylogenetic analysis by Nemec and Brinkhurst (1987), however, indicates that the three latter taxa are not closely related to the *Pristina*/*Pristinella* complex. The (synapomorphic) possession of a proboscis thus appears to support monophyly of *Pristina sensu stricto*, i.e., assuming that the absence of a proboscis in *Pristinella* is not the result of a secondary loss of this character.

The observations on the present material elucidate the importance of including genital features in assessments of naidid morphology and phylogeny. Much work remains until the phylogenetic relationships within the *Pristina*-*Pristinella* complex, as well as within the Naididae as a whole, are resolved.

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